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# RELATIONSHIP OF TREMATODE SPIRORCHID PARASITES AND THEIR EGGS TO THE OCCURRENCE OF FIBROPAPILLOMAS AFFECTING THE GREEN TURTLE (*CHELONIA MYDAS*)

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## INTRODUCTION

Green turtle fibropapillomatosis (GTF) was first described in Florida over fifty years ago by Smith and Coates (1938). Little additional work has been done on this problem until recently when fibropapillomas were noted as common (57% between 1985-1986) in east central Florida (Ehrhart et al. 1991). Similarly, the green turtle in the Hawaiian Islands has also been plagued by GTF during recent years (Balazs 1991).

The etiologic agent for these tumors is still the subject of speculation. One avenue of research is a parasite-tumor relationship. This study stems from the knowledge that trematode eggs from the family Spirorchidae have been found in tissue sections of fibropapilloma tumors in green turtles. Preliminary work has demonstrated numerous (up to several thousand) spirorchid eggs present in a single tumor. This study looked at an array of tumors and attempted to quantify the egg-tumor relationship.

This study utilized the following activities to explore the GTF syndrome:

1. Collection of eight turtles from the wild to examine their tumors.

2. Complete documentation of tumors, including measurements and weights.

3. Digestion of entire tumors to release the eggs present.

4. Counting of eggs from each tumor and separation into type of trematode represented.

5. Selection for euthanization of one or two turtles that could not be released due to injury or extensive tumor formation. These turtles were surveyed for total number of trematode eggs in tumors, as well as adult worms producing eggs in the circulatory system.

6. Survey blood samples from various turtles with and without tumors for spirorchid eggs.

7. Survey normal tissue from turtles with and without tumors for fluke eggs using a punch biopsy.

8. Collection of eggs from tumors made under such conditions that the viability of these eggs could be ascertained. Attempts were made to hatch eggs to the maricidial stages for verification of viability.

9. Test the parasite theory as the possible etiologic agent of these fibropapillomas by recovering trematode eggs and injecting them into normal (non-tumor) and tumored turtles. These turtles were subsequently observed in captivity for tumor formation for 12 months.

## MATERIALS AND METHODS

Turtles were hand captured from Kaneohe Bay, Oahu, Hawaii by personnel of the National Marine Fisheries Service. The tumors were measured and excised prior to the animals release. Turtles found stranded ashore and demonstrated no chance for survival were euthanized for necropsy (Table 1). Animals were dispatched by a humane method using Pentobarbital Sodium.

Tumors were surgically removed from all turtles. Live turtles received a lidocaine nerve block before surgical removal. Most of the live turtle samples consisted of small tumors that did not require sutures after removal. The large tumors from freshly euthanized turtles were cut into smaller pieces to assist in digestion. Tumors were digested in pepsin 1:10,000 from porcine stomach mucosa. Approximately 9.5-10.0 gm pepsin were mixed with 50 cc of water for the digestive process. This concentration was deemed adequate for digestion by trial and error. The tumor tissue was placed in plastic containers with perforated lids and incubated at 40°C for periods of 48-72 hours.

Usually, most of the digestion was complete in 48 hours except for some remaining fibrous tissue. Occasionally some of the larger pieces had to be incubated for longer periods. Incubation times of 4 days did not affect the egg extraction procedure. Spirorchid eggs were incubated and observed for 5 days in this solution with no apparent effect on the ova.

After digestion, the eggs were extracted by passing them through a Flukfinder.® The Flukfinder® (trade mark name by Visual Difference, 5051C Old Pullman Rd., Moscow, ID 83843) consists of a double filtration container with a top stainless steel screen 100 microns in diameter and a bottom screen 40 microns in diameter. It was designed for the extraction of fluke eggs in large animal feces.

The first screen removed large particulate matter, but allowed the fluke eggs to pass through and become trapped by the bottom screen. Flushing the particulate with tap water facilitated the passage of material. Usually, three or four flushes were adequate. The bottom filter was then backwashed into a beaker using a squeeze bottle. This material was placed in test tubes and centrifuged for 5 minutes at 1550 rpm. The top fluid was decanted and the residue placed in a test tube with several drops of 10% Formalin until eggs could be examined and counted.

All tumors collected were measured and weighed prior to processing with the enzyme digestion-filter process. Eggs were then concentrated via centrifugation and examined under a compound microscope for total number of eggs and species represented. Eggs per gram weight of tumor tissue was calculated. Normal tissue was collected by punch biopsy and processed in the same manner as tumor material.

Five turtles captured from Kaneohe Bay were selected for the trematode ova infection studies (Table 2). Four turtles were free of any visible external tumors, while the fifth turtle had numerous tumors present. The tumored turtle was selected as part of the study to determine if those with growths may be sensitized by the infection and perhaps more prone to develop new growths. The turtles were maintained on a diet of previously frozen squid with vitamin/mineral supplements at the National Marine Fisheries Service's Kewalo Research Facility in Honolulu.

A source of viable spirorchid ova was found in the small intestinal serosa of necropsied tumored turtles. Most of the heavily tumored turtles had these present. They appeared as small black spots, approximately 1-2 mm in diameter on the surface of the intestines. Each spot contained a packet of spirorchid ova numbering 100-150 ova. Viability was confirmed by observing miracidial activity within the egg. Eggs were maintained in sea water and continued to be viable for up to one month after collection. The ova packets were injected subcutaneously in the same sites on all of the turtles; that is, left lower eyelid, and the base of left and right front and rear flippers (Figure 1). One turtle (V471) received an additional injection at the tag site of the right front flipper. The injections were divided into three dosages - equal volumes of saline (0.2 cc) were used as a vehicle to carry the ova under the skin. A light dose contained 100-150 eggs, and was injected below the left rear flipper. A medium dose 300-500 eggs was injected in the left lower eyelid. A heavy dose of 1000-1500 eggs was injected at the base of the right front flipper and at the tag site of V471. The site at the base of right rear flipper was injected with eggs that had been killed with 70% alcohol emersion. The site at the base of the left front flipper was injected with eggs that were cleaned in 10% clorox (to eliminate viruses). Both sites received a medium egg dosage. Two control sites (right lower eyelid and cloaca) were injected with saline solution.

All turtles were weighed and measured every two weeks for six months and monthly thereafter. Observations for tumor growth were made every two weeks for six months and then monthly for six months. Total observation time was twelve months.

## RESULTS

A total of 62 tumors were digested and examined from both wild caught (2) and beached, necropsied turtles (6). These tumors were collected from both internal and external sources and ranged in size from 0.2 gm to 347 gm. Egg recovery occurred in 100% of the tumors examined. Egg numbers ranged from less than 1 per gram of tissue to over 845 eggs per gram of tumor tissue.

### Turtle No. 1

A female measuring 58.8 cm in carapace length and weighing 18.6 kg was picked up from the Kailua Canal on Oahu and taken to Sea Life Park on 3/2/92. The turtle was severely emaciated and in poor health. The animal was euthanized for tumor work on 4/1/92. The turtle had 27 tumors; 5 internal and 22 external. Examination of the internal vessels, heart and spleen revealed 167 adult trematodes (71 spirorchid, 96 nonspirorchid). The spirorchid flukes represented three genera, *Learedius*, *Hapalotrema* and *Carettacola* (see Dailey et al. 1991, 1992 and 1993).

The most numerous ova/gram of external tumor tissue was 176 from a 4 gram neck growth. External tumors as a group averaged 60 ova/gram of tissue. Five lung tumors were sampled. One growth had 248 ova/gram. The average number of ova/gram for the five tumors was 145 ova/gram. Normal tissues sampled averaged 6 ova/gram.

The most numerous spirorchid ova per gram of tissue was represented by the species *Learedius learedi* (62%), followed by *Hapalotrema dorsopora* (34%) and *Carettacola hawaiiensis* (4%).

The lung granulomas averaged 122 ova/gram *L. learedi*, 21 ova/gram *H. dorsopora* and 2 ova/gram *C. hawaiiensis*. The external tumors averaged 31 ova/gram *L. learedi*, 27 ova/gram *H. dorsopora* and 3 ova/gram *C. hawaiiensis*. Normal tissue counts were 1.7 ova/gram *L. learedi*, 4.4 ova/gram *H. dorsopora* and 0.2 ova/gram *C. hawaiiensis* (Table 3).

### Turtle No. 2

A female measuring 53.4 cm in carapace length and weighing 20.0 kg was recovered from Kaneohe Marine Corps Air Station, Oahu, Hawaii, and removed to the Kewalo Research Facility on 1/8/92. The turtle was covered with both leeches and barnacles and was in very poor health. The animal was euthanized for tumor work on 4/2/92. The turtle had 20 tumors (11 external, 9 internal). Two hundred and sixty-one adult spirorchid trematodes were recovered from the heart and other internal organs. This turtle had extensive internal tumors. Five liver tumors were sampled as well as one lung tumor and 3 kidney tumors. The liver tumors averaged 35 ova/gram of tissue. The species breakdown was

as follows: *L. learedi* 6.8 ova/gram, *H. dorsopora* 26.6 ova/gram and *C. hawaiiensis* 1.8 ova/gram. The kidney tumors averaged 114.6 ova per gram of tissue. *L. learedi* was represented as 35 ova/gram, *H. dorsopora* 68.3 ova/gram and *C. hawaiiensis* 11.3 ova/gram. The lung granuloma contained 243 ova/gram of tissue. Species differences were *L. learedi* 33 ova/gram, *H. dorsopora* 204 ova/gram and *C. hawaiiensis* 6 ova/gram. Normal tissue contained less than one ova per gram (Table 4).

**Turtle No. 3**

This turtle had no external tumors and was used as a "clean" control animal. This male was found on Maui on 8/5/90 with a spear wound in its head. The turtle was brain damaged and could not be returned to the wild. It was maintained at the Kewalo Research Facility until 4/3/92, at which time it was euthanized for study. It had a carapace length of 47.8 cm and weighed 11.8 kg. Upon examination only one small nodule was found on the lung surface which was not considered a tumor but scar tissue. Five *L. learedi* found in the heart were the only spirorchid flukes recovered from this turtle. Three normal tissue samples were taken from this control animal: the front flipper 113.2 gm, rear flipper 79.2 gm and neck 113.3 gm. A total of 11 ova were recovered from 305.6 grams of tissue. The ova per gram of tissue, as well as the species count, were exceedingly low (see Table 5).

**Turtle No. 4**

This 21.4 kg heavily tumored emaciated turtle measuring 56.7 cm in carapace length was picked up in Kaneohe Bay and kept alive at the Kewalo Research Facility. Two small tumors were selected for study on 6/10/92. The turtle was later euthanized on 8/11/92, and additional tumors were used in the study. This is listed as Turtle Case No. 7. One eye tumor contained 32 ova/gram of tissue. Equal numbers of ova (16) were represented by *L. learedi* and *C. hawaiiensis*. A small shell growth was also selected. It contained 35 ova/gram tissue with *L. learedi* 15 and *H. dorsopora* 20 ova/gram (Table 6).

**Turtle No. 5**

This turtle weighed 17.7 kg and measured 48.2 cm in carapace length. It was captured in Kaneohe Bay on 6/16/92 and released on the same day after being sampled. A small (.4 gm) growth was removed from the right front flipper. This growth contained 5 ova per gram of tissue; all ova were *L. learedi* (Table 7).

**Turtle No. 6**

This turtle weighed 41.8 kg and measured 67.3 cm in carapace length. It was captured in Kaneohe Bay on 6/16/92 and released on the same day after being sampled. A small neck tumor had 32

ova/gram of tissue. *H. dorsopora* were the most numerous with 28 ova/gram. *L. learedi* had 4 and no *C. hawaiiensis* were present (Table 8).

**Turtle No. 7**

This heavily tumored emaciated turtle was collected from Kaneohe Bay, Oahu. The turtle was euthanized 8/11/92. It weighed 20.9 kg and measured 56.9 cm in carapace length. Eight external tumors were sampled, as well as part of the normal right kidney, the entire normal heart and the entire brain (which appeared normal). The external tumors averaged 139 ova/gram of tissue. The species differences were *H. dorsopora* 110.5 ova/gram (79%); *L. learedi* 16.7 (12%) and *C. hawaiiensis* 12 (9%). Normal kidney tissue contained 109 ova/gram with *C. hawaiiensis* 74 ova/gram, *H. dorsopora* 18 and *L. learedi* 17. The heart contained massive amounts of ova, far too many to count. A partial count revealed greater than 604 ova/gram of tissue with *H. dorsopora* being the greatest number of ova/gram (>568) followed by *L. learedi* (>34) and *C. hawaiiensis* greater than two. The entire brain contained 319 ova/gram of tissue. The majority were *H. dorsopora* 227 followed by *L. learedi* 92. *C. hawaiiensis* was not present (Table 9).

**Turtle No. 8**

This 18.2 kg heavily tumored turtle was euthanized for toxicological studies (Aguirre 1993). A glottis tumor was removed and found to have 11 ova/gram of tissue; *H. dorsopora* 9 ova/gram and *L. learedi* two. A 4 gm section of small intestine was sampled in an area where ova packets were visible on the serosa. The evaluation was to determine the species composition of the packets and give some idea as to ova density. A total of 516 ova/gram of tissue was found. The most abundant species was *L. learedi* 448 (87%) followed by *C. hawaiiensis* 50 (10%) and *H. dorsopora* 18 (3%) (Table 10).

**Turtle No. 9**

This 43.6 kg heavily tumored and debilitated turtle measuring 71.3 cm in carapace length was euthanized for toxicological studies (Aguirre 1993). Two small tumors were removed, one from the neck and the other from the right front flipper. They contained 11 and 12 ova/gram respectively; both only harbored *H. dorsopora* ova (Table 11).

**EXPERIMENTAL INFECTIONS**

All turtles demonstrated rapid growth rates and weight gains during the year in captivity (Table 2). However, the tumored turtle (H921) showed less growth and weight gain than the 4 non-tumored turtles. Two turtles developed new tumor growths that were histologically diagnosed as fibropapillomas (V471 and H951).

These growths did not appear to have developed at the injection sites. The tumored turtle (H921) developed a new growth from the cloaca that was saved for histopathology.

**Turtle V471**

During the 12 months of observation, turtle V471 developed a small lump at the control injection site (right lower eyelid) after 3 months. In 5 months, it grew to 5 mm x 3 mm. In 7 months it grew to 9 mm x 4 mm and appeared like a fibropapilloma. In the 8th month, it appeared to have been traumatized and was raw and bleeding. Most of the tumor was missing. The next month (10th) the growth appeared to be coming back and measured 5 mm x 3 mm. After 12 months it measured 6 mm x 4 mm.

During the 11th month, a small 1 mm growth appeared in the left eye (medial canthus). By the 12th month, this growth measured 3 mm x 2 mm.

**Turtle H951**

This turtle developed a small lump on the left eye (medial canthus) after 11 months. During the 12th month it appeared as a definite fibropapilloma and measured 4 mm x 3 mm.

**Turtle H921**

This turtle with tumors developed a new growth that originated from the mucosa of the cloaca. After 8 months, it measured 10 mm x 4 mm and was surgically removed and saved for histopathology. No additional growths were noted.

**Turtle V393**

No growths developed in 12 months.

**Turtle H924**

No growths developed in 12 months.

## DISCUSSION

The cause or causes (pathogen plus environmental insult) responsible for the development of fibropapillomas in green turtles has yet to be determined. Recently, Jacobson et al. (1991) reported the presence of a herpesvirus in two cases of GTF. However, these researchers were unable to fulfill Koch's postulate and determine if a causal relationship existed between the herpesvirus demonstrated in fibropapillomas. Of the 73 fibropapillomas he examined from Hawaiian green turtles, evidence of herpesvirus infection, such as formation of intranuclear inclusions, was not observed in any of the sections examined. Jacobson (1992) states that trematodes eggs from the family

Spirorchidae were seen more often within the dermal capillaries of cutaneous fibropapillomas of Hawaiian caught turtles as compared to those from Florida. He indicates that trematode eggs were few in observed sections of fibropapillomas and it may take multiple sections of a given tumor to clearly demonstrate the presence or absence of eggs.

Pollutants have been implicated as a possible related cause of fibropapillomas. A recent investigation by Aguirre (1993) on environmental pollutants present in Hawaiian green turtles with fibropapillomas yielded negative results.

The present study in Hawaii looked at the total number of eggs in the entire tumor through digestion, and which species of spirorchid trematode were involved. The results indicate clearly that 100% of all tumors found during this study, no matter how small or where they were located, contained spirorchid trematode eggs. Not only did all tumors contain eggs, but usually in massive number. A tumor on the cornea of Turtle No. 2 contained 845 ova/gram of tissue. Even the smallest tumors contained a significant number of ova. A 0.6 gm shell tumor had 310 ova/gram, and a 0.5 gm eye tumor had 32 ova/gram. Internal tumors also contained large numbers of ova. A lung tumor (2.5 gms) contained 243 ova/gram. The average lung granuloma contained 161 ova/gram. The liver and kidneys were also tumored in some of the turtles. The liver tumors averaged 35 ova/gram, while the kidney tumors averaged 115 ova/gram. The significance of this information has yet to be determined. However, we feel it establishes a possible direct correlation between GTF and spirorchid trematode infections.

Pathogenesis caused by eggs of trematodes infecting the circulatory systems of various hosts has been well documented (Schmidt and Roberts, 1989). In the genus *Schistosoma* it is stated "the most serious damage is done by the eggs of all three species". The eggs lodged in the venules and submucosa act as foreign bodies and cause inflammatory reactions with leukocytic and then fibroblastic infiltration. These finally become fibrous nodules (Schmidt and Roberts, 1989). Such nodules were histologically identified as fibromas in our lung tumors and also appeared as small white nodules in the intestinal mucosa. The various tissues (internal vs epidermis and dermis) may react and produce different appearing growths both histologically and visually.

Three species of blood flukes, *L. learedi*, *H. dorsopora* and *C. hawaiiensis*, which are found in large numbers in Hawaiian green turtles, occupy similar habitat niches to members of the genus *Schistosoma*. This is especially true with the distribution of eggs throughout the body. The finding in this study of a high egg count in brain tissue (319 ova/gm in Turtle No. 7) demonstrated the extent to which these eggs are spread in the circulatory system. The effect on the turtle of such large

numbers of ova in the brain is unknown. The opportunity for these eggs to lodge in vessels and act as foreign bodies that could cause fibrotic infiltration are myriad. Even though the injection of ova into normal turtle tissue did not result in tumor formation at the injection site after 12 months, additional investigation should be pursued. Perhaps environmental factors may be involved, as well as stress, parasite load or a combination of the above. We do know that the so called "normal" turtles utilized for this study, were infected with all three spirorchid species. This was determined from fecal ova observations. However, ova could not be demonstrated in the blood samples taken from three heavily parasitized turtles. Ova were also present in normal epidermal tissue, but in small numbers. Large numbers of ova were found in normal kidney and heart tissue of tumored turtles. We feel the findings to date in this study of eggs occurring in 100% of all tumors and massive numbers throughout the body warrant continued research.

Although the transmission cycle of the marine species in the family Spirorchidae has not been elucidated, the cycle of *Spirorchis elegans*, a species that lives in the blood vessels of freshwater turtles, is known (Goodchild and Kirk, 1960). In the *S. elegans* cycle the cercaria are shed from a snail and penetrate the mucous membranes of the eye, nose, mouth and cloaca of turtles. Recent studies on the snails inhabiting the feeding grounds of Hawaiian green turtles has been negative to date for spirorchid trematode larvae (Dailey, pers. observ.). This observation may indicate that the life cycle of marine spirorchids infecting sea turtles may use a non snail intermediate host. Køie (1982) has found the cercaria of *Aporocotyle simplex*, a sanguinocolid blood fluke of fishes, uses a polychaete annelid for the intermediate host. An increase in the numbers of parasites present in inshore turtle feeding areas could account for an increase in fibropapillomas. This presents a direction for future research on the life cycle of green turtle spirorchid parasites.

If trematode ova, perhaps in synergism with some other factor, are in fact the etiologic agents, the lesions would then represent a non-neoplastic host response, characterized by an exuberant cutaneous, foreign body fibrosis associated with papillary epidermal hyperplasia.

Preliminary work has begun on isolating the protein that causes the host to react to the egg. An excellent source of ova for this work was found in the serosa of tumored turtles. These appeared as small "black dots". The "escape" route of the spirorchid ova to the environment is through the feces. Perhaps a heavy parasite load contributes to this accumulation of ova in the serosa, as it is not generally noted in lightly or non-tumored turtles. This is the first report of intestinal ova in Hawaiian turtles. Eggs of these spirorchid trematodes can be kept alive for months in sea water. These eggs (miracidial

larval stage in the egg observed) have been used for this initial protein work. Findings to date indicate that a protein is present in the sterile saline solution in which these eggs have been cultured (Dr. J. Sakanari, Univ. of Calif., San Francisco, pers. comm.). Additional work is needed to find the specific protein involved and to prove it is parasite induced rather than from the host turtle. Once isolated, this protein can be collected and tested for fibropapilloma production.

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Table 1. SUMMARY OF TURTLES SAMPLED

| Turtle No. | Identification | Weight (kg) | Carapace length (cm) | Sex |
|---|---|---|---|---|
| 1 | J. C. | 18.6 | 58.8 | F |
| 2 | R. C. | 20.0 | 53.4 | F |
| 3 | Maui (Speared in head) | 11.8 | 47.8 | M |
| 4[a] | Y-88 | 21.4 | 56.7 | F |
| 5 | H-800 | 17.7 | 48.2 | - |
| 6 | H-807 | 41.8 | 67.3 | - |
| 7[b] | Y-88 | 20.9 | 56.9 | F |
| 8 | HI92-46 | 18.2 | 56.0 | F |
| 9 | HI92-48 | 43.6 | 71.3 | M |

[a]Sampled alive 6/10/92
[b]Necropsy 8/11/92

Table 2. GROWTH RATES AND WEIGHT GAINS OF EXPERIMENTAL TURTLES IN CAPTIVITY

| Turtle | Initial | | 12 month period | | | |
|---|---|---|---|---|---|---|
| | Carapace length (cm) | Weight (kg) | Carapace length (cm) | Gain | Weight (kg) | Gain |
| H924 | 42.2 | 10.0 | 51.9 | +9.7 | 20.5 | +10.5 |
| V471 | 42.2 | 8.6 | 50.0 | +7.8 | 18.2 | +9.6 |
| H951 | 41.7 | 10.9 | 46.9 | +5.2 | 16.4 | +5.5 |
| V393 | 40.7 | 10.0 | 45.6 | +4.9 | 14.5 | +4.5 |
| H921 (tumors) | 42.9 | 11.8 | 46.0 | +3.1 | 15.0 | +3.2 |

Table 3. SPIRORCHID EGG COUNTS FOUND IN TUMORS AND NORMAL TISSUE OF TURTLE NO. 1

| Location | Length Size (mm) | Tissue Weight (g) | Number of ova by species L. l.[a] (ova/g) | H. d.[b] (ova/g) | C. h.[c] (ova/g) | Total No. ova | Total ova/g |
|---|---|---|---|---|---|---|---|
| | | | Turtle No. I (tumors) | | | | |
| Cornea | 11 | 0.5 | 18 (36) | 18 (36) | 0 | 36 | 72 |
| L. eye | 40 | 5.5 | 392 (71) | 49 (9) | 2 (.4) | 443 | 82.4 |
| L. cornea | 30 | 4.0 | 384 (96) | 0 | 4 (l) | 388 | 97 |
| R. eye ring | 30 | 3.0 | 0 | 47 (16) | 0 | 47 | 16 |
| R. eye | 10 | 0.5 | 0 | 8 (16) | 0 | 8 | 16 |
| L. jaw | 25 | 1.7 | 64 (38) | 58 (34) | 9 (5) | 131 | 77 |
| R. jaw | 23 | 1.0 | 51 (51) | 47 (47) | 3 (3) | 101 | 101 |
| Neck | 50 | 20.0 | 84 (4) | 82 (4) | 14 (.7) | 180 | 8.7 |
| Neck | 25 | 4.0 | 312 (78) | 343 (86) | 46 (12) | 701 | 176 |
| Neck | 40 | 20.0 | 968 (48) | 920 (46) | 212 (11) | 2100 | 105 |
| R. Lung | 5 | 0.2 | 23 (115) | 2 (10) | 0 | 25 | 125 |
| L. Lung | l5 | 1.0 | 113 (113) | 6 (6) | 4 (4) | 123 | 123 |
| L. Lung | 30 | 8.5 | 1831 (215) | 270 (32) | 9 (1) | 2110 | 248 |
| L. Lung | 30 | 7.5 | 1230 (164) | 416 (55) | 28 (4) | 1674 | 223 |
| R. Lung | 35 | 13.4 | 48 (4) | 12 (.9) | 7 (.5) | 67 | 5.4 |
| R. F. Flip | 75 | 94.0 | 264 (3) | 42l (4) | 197 (2) | 882 | 9 |
| R. F. Flip | 100 | 180.0 | 330 (2) | 996 (6) | 652 (4) | 1978 | 11 |
| R. F. Flip | 40 | 20.5 | 15 (.7) | 286 (14) | 178 (9) | 479 | 23.7 |
| L. F. Flip | 15 | 1.0 | 57 (57) | 26 (26) | 1 (1) | 84 | 84 |
| L. F. Flip | 100 | 136.0 | 128 (.9) | 1344 (10) | 1152 (8) | 2624 | 18.9 |
| R. R. Flip | 14 | 1.0 | 45 (45) | 38 (38) | 3 (3) | 86 | 86 |
| R. R. Flip | 15 | 1.0 | 49 (49) | 40 (40) | 6 (6) | 95 | 95 |
| R. R. Flip | 25 | 1.5 | 57 (38) | 28 (19) | 12 (8) | 97 | 65 |
| R. R. Flip | 14 | 1.0 | 3 (3) | 24 (24) | 0 | 27 | 27 |
| L. R. Flip | 10 | 0.6 | 8 (13) | 16 (27) | 0 | 24 | 40 |
| L. R. Flip | 10 | 0.6 | 12 (20) | 20 (33) | 1 (2) | 33 | 55 |
| Cloaca | 10 | 0.5 | 0 | 26 (52) | 0 | 26 | 52 |
| Normal L. F. | 30 | 1.3 | 0 | 11 (8) | 1 (.7) | 12 | 8.7 |
| Normal Neck | 25 | 5.0 | 1 (.2) | 1 (.2) | 0 | 2 | 0.4 |
| Normal L. R. | 30 | 4.0 | 18 (5) | 18 (5) | 0 | 36 | 10 |
| Filtered Blood | | 25 cc | 0 | 0 | 0 | 0 | 0 |
| TOTAL ova/g tissue | | | 1269.6 | 704.1 | 86.3 | | |
| PERCENT of total ova/g tissue | | | 62% | 34% | 4% | | |

[a] *L. learedi*
[b] *H. dorsopora*
[c] *C. hawaiiensis*

*Ova per gram of tissue rounded to nearest whole number except if less than one, it was carried as fraction.

Table 4. SPIRORCHID EGG COUNTS IN TUMORS AND NORMAL TISSUE OF TURTLE NO. 2

| Location | Length Size (mm) | Tissue Weight (g) | Number of ova by species: L. l.[a] (ova/g) | H. d.[b] (ova/g) | C. h.[c] (ova/g) | Total No. ova | Total ova/g |
|---|---|---|---|---|---|---|---|
| | | | Turtle No. 2 (tumors) | | | | |
| R. cornea | 25 x 25 | 3.5 | 2210 (631) | 731 (209) | 17 (5) | 2958 | 845 |
| R. eye | 25 x 10 | 2.0 | 212 (106) | 117 (58) | 0 | 329 | 164 |
| L. eye | 20 x 10 | 1.0 | 29 (29) | 16 (16) | 3 (3) | 48 | 48 |
| L. lung | 20 x 20 | 2.5 | 83 (33) | 510 (204) | 15 (6) | 608 | 243 |
| Liver | 25 x 5 | 1.5 | 9 (6) | 39 (26) | 0 | 48 | 32 |
| Liver | 75 x 1.5 | 133.0 | 2047 (15) | 11,935 (90) | 403 (3) | 14385 | 108 |
| Liver | 65 x 65 | 144.0 | 274 (2) | 136 (1) | 132 (1) | 542 | 4 |
| Liver | 30 x 30 | 18.5 | 91 (5) | 255 (14) | 79 (4) | 425 | 23 |
| Liver | 25 x 20 | 3.2 | 18 (6) | 6 (2) | 2 (1) | 26 | 9 |
| R. kidney | 80 x 60 | 74.0 | 1102 (15) | 833 (11) | 84 (1) | 2019 | 27 |
| R. kidney | 20 x 20 | 5.5 | 212 (39) | 61 (11) | 7 (1) | 280 | 51 |
| R. kidney | 90 x 60 | 69.0 | 3528 (51) | 12600 (183) | 2226 (32) | 18354 | 266 |
| R. F. flip | 10 X 10 | 0.8 | 2 (3) | 1 (1) | 0 | 3 | 4 |
| R. F. flip | 100 x 80 | 126.0 | 328 (3) | 416 (3) | 72 (1) | 816 | 7 |
| R. F. flip | 75 x 50 | 68.0 | 79 (1) | 96 (1) | 17 (.2) | 192 | 2.8 |
| L. F. flip | 100 x 80 | 137.0 | 632 (5) | 746 (5) | 57 (.4) | 1435 | 10.4 |
| R. R. flip | 25 x 20 | 3.0 | 22 (7) | 132 (44) | 12 (4) | 166 | 55 |
| R. R. flip | 15 x 4 | 0.7 | 6 (9) | 49 (70) | 3 (4) | 58 | 83 |
| L. R. flip | 85 x 60 | 69.0 | 58 (.8) | 339 (5) | 3 (.04) | 400 | 5.8 |
| L. R. flip | 160 x 90 | 347.0 | 1363 (4) | 18588 (54) | 399 (1) | 20350 | 59 |
| Normal neck | 40 x 30 | 4.5 | 2 (.4) | 1 (.2) | 0 | 3 | .6 |
| Normal F. flip | | 25.0 | 7 (.3) | 21 (.8) | 0 | 28 | 1.1 |
| Normal R. flip | 40 x 30 | 18.0 | 2 (.1) | 2 (.1) | 0 | 4 | .2 |
| Filtered blood | | 10 cc | 0 | 0 | 0 | 0 | 0 |
| TOTAL ova/g tissue | | | 971.6 | 1009.1 | 67.6 | | |
| PERCENT of total ova/g tissue | | | 47% | 49% | 3% | | |

[a] L. learedi
[b] H. dorsopora
[c] C. hawaiiensis

Table 5. SPIRORCHID EGG COUNTS FOUND IN NORMAL TISSUE OF TURTLE NO. 3

| Location | Length Size (mm) | Tissue Weight (g) | Number of ova by species L. l.[a] (ova/g) | H. d.[b] (ova/g) | C. h.[c] (ova/g) | Total No. ova | Total ova/g |
|---|---|---|---|---|---|---|---|
| | | | Turtle No. 3 (normal) | | | | |
| Neck | -- | 113.3 | 1 | 0 | 0 | 1 | <1 |
| F. flip | -- | 113.2 | 0 | 2 | 0 | 2 | <1 |
| R. R. flip | -- | 79.2 | 2 | 6 | 0 | 8 | <1 |

[a] L. learedi
[b] H. dorsopora
[c] C. hawaiiensis

Table 6. SPIRORCHID EGG COUNTS FOUND IN TUMORS OF TURTLE NO. 4

| Location | Length Size (mm) | Tissue Weight (g) | Number of ova by species L. l.[a] (ova/g) | H. d.[b] (ova/g) | C. h.[c] (ova/g) | Total No. ova | Total ova/g |
|---|---|---|---|---|---|---|---|
| | | | Turtle No. 4 | | | | |
| Eye | 15 x 5 | .5 | 8 (16) | 8 (16) | 0 | 16 | 32 |
| Shell | 20 x 18 | 1.5 | 23 (15) | 30 (20) | 0 | 53 | 35 |
| TOTAL ova/g tissue | | | 31 | 38 | 0 | | |
| PERCENT of total ova/g tissue | | | 45% | 55% | | | |

[a] L. learedi
[b] H. dorsopora
[c] C. hawaiiensis

Table 7. SPIRORCHID EGG COUNTS FOUND IN TUMOR OF TURTLE NO. 5

| Location | Length Size (mm) | Tissue Weight (g) | Number of ova by species | | | Total No. ova | Total ova/g |
|---|---|---|---|---|---|---|---|
| | | | L. l.[a] (ova/g) | H. d.[b] (ova/g) | C. h.[c] (ova/g) | | |
| | | | Turtle No. 5 | | | | |
| R. F. Flip | 10 x 5 | .4 | 2 (5) | 0 | 0 | 2 | 5 |

[a] L. learedi
[b] H. dorsopora
[c] C. hawaiiensis

Table 8. SPIRORCHID EGG COUNTS FOUND IN TUMOR OF TURTLE NO. 6

| Location | Length Size (mm) | Tissue Weight (g) | Number of ova by species | | | Total No. ova | Total ova/g |
|---|---|---|---|---|---|---|---|
| | | | L. l.[a] (ova/g) | H. d.[b] (ova/g) | C. h.[c] (ova/g) | | |
| | | | Turtle No. 6 (tumor) | | | | |
| Neck | 20 X 12 | 2.1 | 8 (4) | 59 (28) | 0 | 67 | 32 |

[a] *L. learedi*
[b] *H. dorsopora*
[c] *C. hawaiiensis*

Table 9. SPIRORCHID EGG COUNTS FOUND IN TUMORS AND NORMAL TISSUE OF TURTLE NO. 7

| Location | Length Size (mm) | Tissue Weight (g) | Number of ova by species: L. l.[a] (ova/g) | H. d.[b] (ova/g) | C. h.[c] (ova/g) | Total No. ova | Total ova/g |
|---|---|---|---|---|---|---|---|
| R. F. flip | 25 x 25 | 5.0 | 60 (12) | 410 (82) | 5 (1) | 475 | 95 |
| R. F. flip | 130 x 50 | 122.0 | 3930 (32) | 6488 (53) | 436 (4) | 10854 | 89 |
| R. F. flip | 70 x 25 | 19.5 | 72 (4) | 846 (43) | 246 (13) | 1164 | 60 |
| R. F. flip | 30 x 20 | 8.5 | 100 (12) | 1196 (141) | 124 (15) | 1420 | 168 |
| L. F. flip | 25 x 15 | 4.0 | 90 (23) | 450 (113) | 0 | 540 | 136 |
| L. R. flip | 30 x 25 | 8.5 | 160 (19) | 910 (107) | 125 (15) | 1195 | 141 |
| R. R. flip | 20 x 10 | 2.2 | 16 (7) | 208 (95) | 29 (13) | 253 | 115 |
| Shell Edge | | .6 | 15 (25) | 150 (250) | 21 (35) | 186 | 310 |
| Percent of total ova/g tissue | | | 134 (12%) | 884 (79%) | 96 (90%) | | |
| Normal tissue | | | | | | | |
| R. kidney | | 30.0 | 496 (17) | 544 (18) | 2224 (74) | 3264 | 109 |
| *Heart | | 77.5 | 2650 (34+) | 43999 (568+) | 159 (2+) | 46808 | 604+ |
| Entire brain | | 4.0 | 367 (92) | 906 (227) | 0 | 1273 | 319 |
| Filtered blood | | 10 cc | 0 | 0 | 0 | 0 | 0 |

[a] L. learedi
[b] H. dorsopora
[c] C. hawaiiensis

*Counts too high to complete

Table 10. SPIRORCHID EGG COUNTS FOUND IN TUMORS OF TURTLE NO. 8

| Location | Length Size (mm) | Tissue Weight (g) | Number of ova by species: L. l.[a] (ova/g) | H. d.[b] (ova/g) | C. h.[c] (ova/g) | Total No. ova | Total ova/g |
|---|---|---|---|---|---|---|---|
| | | | Turtle No. 8 | | | | |
| Glottis | - | 3.2 | 6 (2) | 28 (9) | 0 | 34 | 11 |
| Sm. Intestine (egg packets) | - | 4.0 | 1790 (448) | 70 (18) | 200 (50) | 2060 | 516 |

[a] L. learedi
[b] H. dorsopora
[c] C. hawaiiensis

Table 11. SPIRORCHID EGG COUNTS FOUND IN TUMORS AND NORMAL TISSUE OF TURTLE NO. 9

| Location | Length Size (mm) | Tissue Weight (g) | Number of ova by species: L. l.[a] (ova/g) | H. d.[b] (ova/g) | C. h.[c] (ova/g) | Total No. ova | Total ova/g |
|---|---|---|---|---|---|---|---|
| | | | Turtle No. 9 | | | | |
| Neck | - | 1.5 | 0 | 16 (11) | 0 | 16 | 11 |
| R. Front | - | .5 | 0 | 6 (12) | 0 | 6 | 12 |

[a] *L. learedi*
[b] *H. dorsopora*
[c] *C. hawaiiensis*

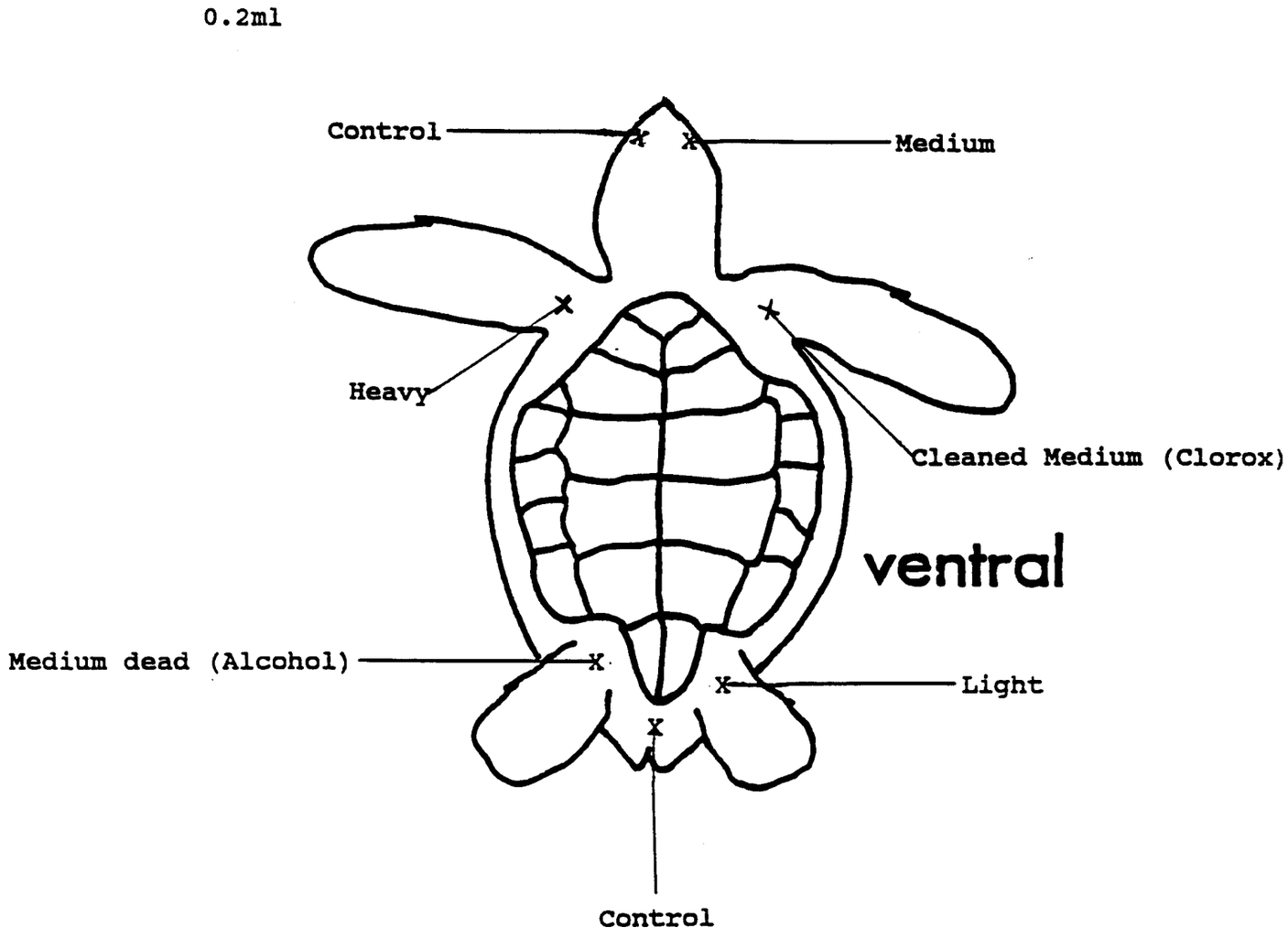


FIGURE 1. Injection sites on experimental turtles (ventral view)